

THE SYMBIOTIC EFFICIENCY OF  
SOME PEANUT CULTIVARS AND  
THEIR INTERACTION WITH STRAINS OF RHIZOBIUM SPP.

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## ABSTRACT

A series of greenhouse and growth room tests were conducted to investigate the interactions between strains of Rhizobium spp. and cultivars of peanut (Arachis hypogea (L.)).

Two preliminary greenhouse studies, Experiments 1 and 2, involved testing five and seven strains of rhizobia respectively on two cultivars of peanut. Experiment 1 identified a strain (TAL 236) which was significantly better than other effective strains on both cultivars (Florida Giant and Starr). Data from the effective strains (those which reduced acetylene) indicated that cultivar Florida Giant fixed more nitrogen in early growth than Starr. Both cultivars had similar growth potential when provided  $\text{NH}_4\text{NO}_3$ . Experiment 2 showed significant strain by cultivar interactions among effective strains. Burpee Spanish yielded significantly more than Florunner when inoculated with strain AH8 but significantly less than Florunner when both were inoculated with strain T-1. This interaction emphasized the danger in assuming that strains selected for high efficiency on one cultivar of peanut will perform in a similar fashion on another. Strain TAL 1000 proved to be better than the other effective strains when data from both cultivars were considered.

Observations of plants during Experiments 1 and 2 indicated that plants inoculated with strains TAL 236 and TAL 1000 underwent greening of foliage earlier than those plants inoculated with less effective strains. A growth room experiment (Experiment 3) was undertaken to determine whether the earlier greening of foliage by

the more effective host-strain combinations were related to the time required for nodule formation. Experiment 3 revealed a significant relation between host seed size and the time to nodule formation. However, the time to nodule formation could not be related to the degree of symbiotic effectiveness of host-strain combinations as determined in Experiments 1 and 2.

Effective strains TAL 1000, TAL 236, and TAL 309 were selected to test the symbiotic effectiveness of 12 cultivars of peanut in Experiment 4. Differences between cultivars and significant strain by cultivar interactions were revealed. The yields of the cultivars were more uniform when provided  $\text{NH}_4\text{NO}_3$  than when relying on the symbiosis as a nitrogen source. Some cultivars' average symbiotic yields were over 80% of their respective yields when supplied with mineral N. Other less efficient cultivars yielded only 50% of their mineral N controls. Cultivars inoculated with TAL 1000 generally had greater yields than those inoculated with strains TAL 309 or TAL 236.

These tests demonstrated the specific host by strain interactions involved in the peanut-Rhizobium symbiosis. An effective symbiosis is both host and strain determined. Strains which had been shown to be highly effective on more than one cultivar in preliminary tests did not always result in a highly effective symbiosis with other cultivars.

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## INTRODUCTION

Nations with rising populations, especially those in the tropics where per capita protein consumption is currently in a marginal state (U.N.F.A.O., 1973), face a dilemma in their attempt to produce adequate amounts of protein for human consumption. To meet this growing protein demand, there will either have to be a shift of scarce resources into the production of energy-expensive nitrogenous fertilizer or a greater reliance on biologically-fixed nitrogen. The extent to which the latter method can substitute for industrially produced nitrogen is a function of the world's technological ability to manipulate and utilize nitrogen of biological origins. The legume-Rhizobium symbiosis will be an important part of the effort to harness biologically-fixed nitrogen.

Of all legumes grown in the tropics, the peanut (Arachis hypogea) is, without doubt, the most extensively grown, accounting for 52% of total grain legume production (Rachie, 1974). Considering the peanut's importance both as a pulse and as an oil seed for industry or export, there is a relative lack of research directed to improving the peanut-Rhizobium symbiosis.

## REVIEW OF LITERATURE

One possible explanation for the paucity of material concerning the peanut-Rhizobium symbiosis is the peanut's promiscuous nodulating behavior with cowpea type Rhizobium organisms (Gaur, 1974; Vincent, 1974; Dart, 1974) which makes it difficult to demonstrate a clear

response to field inoculation. Some investigations have been directed toward screening numerous strains of the microsymbiont for invasiveness and effectiveness on peanut (Weaver, 1975; Diatloff and Langford, 1975; Van der Merwe, et al., 1974). Few of the trials involved in these studies demonstrated a response to field inoculation even though host recovery of the inoculant strain was, at times, substantial. The presence of highly effective native rhizobia was postulated as an explanation for this phenomenon.

While the microsymbiont has received considerable attention in the past in attempts to classify Rhizobium species differences (Baldwin and Fred, 1929; Graham, 1976), or to determine those parameters correlated with Rhizobium effectiveness, persistence, and competitiveness (Bohlool and Schmidt, 1973; Weber and Miller, 1972; Thompson, et al., 1974; Roughley, et al., 1976; Pinto, et al., 1974), little emphasis has been placed on examining the host effects on these various aspects of the symbiosis. It seems probable that many factors of the symbiosis are host determined, and may therefore be suitable for manipulation.

The problem of being able to introduce an effective strain into a soil environment and subsequently obtain an inoculation response in soils with a large Rhizobium population suggests at least four plausible approaches to increasing nitrogen fixation:

- 1) Screening and genetic manipulation of the bacteria for superior competitiveness and effectiveness;
- 2) Finding host genotypes that are capable of fixing increased amounts of nitrogen with a broad spectrum of Rhizobium



strains;

- 3) Maximizing the effects of better mineral nutrition; and
- 4) Obtaining host selectivity for efficient strains and resistance to nodulation by ineffective Rhizobium strains present in the soil.

Examination of peanut genotypes for their symbiotic capacities with broad spectrum strains of Rhizobium has not been undertaken. Demonstration of variation in either effectiveness or invasiveness of peanut cultivars in combination with a number of Rhizobium warrants some effort. As new cultivars are developed for commercial use, the possibility always exists that without proper screening, cultivars with low nitrogen fixing abilities will be released. Awareness of cultivar-strain specificity may avoid the possibility that a commercial inoculant strain is used on a cultivar for which it is either non-invasive or ineffective. These two possibilities have occurred: strain CB1809 is ineffective on soybean cultivar Hardee (Diatloff and Langford, 1975); and Rhizobium trifolii strains TA 1 and UNZ 29 were non-invasive on Trifolium subterraneum cv. Woogenellup (Gibson, et al., 1976).

Many researchers concerned with the legume-Rhizobium symbiosis consider that many important factors of the symbiosis are host determined. This review of literature will examine and discuss works that pertain to various aspects of host influences on the symbiosis.

Genotypic variation in nitrogen fixation has been demonstrated for: Pisum sativa (Lie, et al., 1976); Medicago sativa (Gibson,

1962); Glycine max (Boyer and Bond, 1942); Trifolium pratense (Nutman and Read, 1952). The most revealing work was that done by Gibson (1962) on Medicago sativa. Fifteen cultivars were examined with six known effective strains of Rhizobium meliloti. Data showed that the mean yields for cultivars were more variable than the mean yields of rhizobial treatments. Yields of all cultivars were similar when supplied with  $\text{NH}_4\text{NO}_3$  indicating that the host varieties all had a similar early yield potential. Strain by variety interaction were also shown to be significant. J. C. Burton has shown a two fold difference in symbiotic nitrogen accumulation with cowpea genotypes inoculated with a wide range of Rhizobium spp.

Symbiotic capacity of host genotypes can be inherited. Crosses between high fixing varieties of Medicago sativa by low fixing varieties yielded host types with an intermediate capacity. This intermediate symbiotic response was independent of general genetic yield potential when given mineral nitrogen (Gibson, 1962).

Workers in Minnesota (Seetin and Barnes, 1977) claim to have doubled nitrogen fixation in alfalfa through a program of selection and breeding. Nutman (1961) demonstrated an increase in fixation in  $F_1$  hybrids of Trifolium subterraneum but was unable to sustain the increase through the  $F_4$ . Duhigg, et al. (1978) have demonstrated wide variability in symbiotic capacity within the alfalfa cultivar 'Mesilla.' Crosses between plants identified as high fixing types had dry weight increases of 57% above the cultivar mean. A multiple strain inoculum was used in the selection process.

Genotypes showing extreme invasive specificity within a cross

inoculation group would facilitate the introduction of a preferred strain of rhizobia into the soil. This type of host characteristic is not frequently observed; however, some discriminating lines such as Trifolium subterraneum cv. Woogenellup do exist (Gibson, et al., 1976).

A non-nodulating characteristic with many strains has been observed for some species: Trifolium pratense (Nutman, 1946); Glycine max (Williams and Lynch, 1954); Pisum sativa (Lie, et al., 1976). Both dominant and recessive genes have been identified as being responsible for the non-nodulating character. Genes for non-nodulation in Pisum sativa were found to be temperature specific (Lie, et al., 1976). It has not been demonstrated whether these genes for invasive specificity can be exploited to increase nitrogen fixation.

Observations concerning the host effects upon the competitive abilities of a mixed Rhizobium population have been made for: Glycine max (Caldwell and Vest, 1968); Trifolium spp. (Vincent and Waters, 1953); Trifolium subterraneum (Roughley, et al., 1976). Robinson (1969) claims, on the basis of his studies with subterraneum clover, that hosts distinguish between effective and ineffective strains and favor the former in nodule formation. Vincent and Waters' data (1953) contradict this finding. The mechanism involved in this phenomenon is not clear. Inheritance of host selectivity for particular strains has not been examined. Whether plants can be developed that select certain desirable soil or inoculum strains for nodulation is not known.

Resistance to infection by single strains has been shown on hosts that normally nodulate with other strains (Lie, et al., 1976). Incorporation of plant resistance to infection by known ineffective soil strains may result in increased nitrogen fixation.

Investigation into either host resistance to infection by ineffective strains or host selectivity of more desirable strains implies that the presence of both effective and ineffective nodules reduces nitrogen fixation below that produced with effective nodules alone. The quantification of this relationship has not been undertaken.

It seems probable that the most productive avenue to increasing nitrogen fixation with promiscuous tropical legumes would appear to be selection of varieties that have a high symbiotic capacity when inoculated with a wide range of rhizobial strains. This study was undertaken to examine this concept for cultivated varieties of peanut. Rather than using large numbers of strains to test each cultivar, a pre-selection process was conducted on strains to ensure their effectiveness with a minimum number of host varieties.

## CHAPTER I

### EXPERIMENT 1: THE SYMBIOTIC EFFECTIVENESS OF FIVE STRAINS OF RHIZOBIUM SPP. WITH TWO CULTIVARS OF PEANUT (ARACHIS HYPOGEA (L.))

#### ABSTRACT

A greenhouse pot experiment was conducted to test the symbiotic effectiveness of two cultivars of peanut (Arachis hypogea (L.)) with five strains of Rhizobium spp. and to observe the suitability of a subirrigation growth system for use in Rhizobium strain testing. The two cultivars, Florida Giant and Starr, were either inoculated with each of five strains of Rhizobium spp. or given 450 mg of mineral N.

Total nitrogen in shoots indicated that there were significant differences among rhizobial strains, cultivars, and significant strain by cultivar interactions.

Acetylene reduction data were not highly correlated with shoot weights or shoot N; however, results did indicate that only three strains (TAL 309 (CB756), TAL 236, TAL 170 176 A23) were capable of reducing acetylene with these two cultivars.

Significant cultivar differences were found in the partitioning of dry matter. The ratio of shoot weight to root weight was larger for the higher yielding cultivar, Florida Giant. Shoot to root ratios were positively correlated with shoot N.

It was observed that strain treatment which reduced acetylene tended to have larger nodules. Nodule number was not related to

effectiveness.

The subirrigation growth system employed in this experiment provided satisfactory control of contamination by extraneous strains of Rhizobium. No uninoculated plants reduced acetylene. No significant diffusion of N into the system occurred from pots given  $\text{NH}_4\text{NO}_3$ .

### INTRODUCTION

The peanut is, without doubt, the most important legume in the tropics; accounting for 52% of total grain legume production (Rachie, 1974). Despite the peanut's importance both as a pulse and as an oil seed for industry and export, there is a relative lack of research directed to improving the peanut-Rhizobium symbiosis.

There have been numerous investigations undertaken to test various strains of Rhizobium for effectiveness on peanut. While greenhouse differences in strain performance are demonstrated (Weaver, 1974; Diatloff and Langford, 1975; Allen and Allen, 1940) few works reported in the literature have indicated that a field response to inoculation with selected strains is possible in the tropics (Diatloff and Langford, 1975; Van der Merwe, et al., 1974; Subba Rao, 1976). Weaver (1974) showed that many peanut Rhizobium from Texas were not as effective as selected strains in laboratory trials yet a yield response to inoculation with selected strains could not be demonstrated in the field (Weaver, personal communication, Texas A & M University). Hickey, et al. (1974) obtained a large response to inoculation of peanut grown in newly cultivated

sands of Florida. Bajpai, et al. (1974) reported a 21% increase in peanut yield with inoculation in India. It appears that a response to inoculation is possible only in special situations. The lack of inoculation response may be due to either larger numbers of invasive Rhizobium in the soil (Caldwell and Vest, 1970), the level of effectiveness of the native strains (Diatloff and Langford, 1975), or adequate soil nitrogen.

The peanut's promiscuous nature (Gaur, et al., 1974; Dart, 1974) and the prevalence of peanut-invasive, cowpea-type Rhizobium in the tropics compound the difficulty in demonstrating a response to field inoculation.

One approach to increasing nitrogen fixation in the peanut may, therefore, involve the selection of host genotypes that fix large amounts of nitrogen with a number of strains of Rhizobium. Some workers have demonstrated this concept for other species of legumes: Medicago sativa (Gibson, 1962; Seetin and Barnes, 1977); Vigna unguiculata (Burton, unpublished); Vicia faba (El-Sherbeeney, et al., 1977).

Gibson (1962) has demonstrated the existence of rhizobial strain by host variety interactions for nitrogen fixation efficiency. Testing cultivars for their nitrogen fixation capacities would be more revealing if the strains used in the test were broad spectrum. This would reduce the possibility that low cultivar performance was strain determined.

By testing strains of Rhizobium with two cultivars of peanut, this study was designed to identify effective strains that would

perform well on both cultivars with minimal strain by cultivar interactions. These strains then provide a basis for testing the symbiotic effectiveness of many additional cultivars in Experiment IV.

Many growth systems have been developed for plant-Rhizobium effectiveness tests. Some of these were the result of pasture legume research. Systems such as tube culture and modified Leonard jars (Vincent, 1970) are either too limiting to growth or require excessive time for construction and maintenance for testing large seeded grain legumes. An alternative system has been proposed by Weaver (1975) that has larger growth capacities and reduced time requirements. The growth system evaluated in this study is a modification of one described by Weaver. An additional objective of this study will therefore be to evaluate such a system for capacity and uniformity of peanut growth and for bacteriological control.

## MATERIALS AND METHODS

### Growth System and Plant Culture

A subirrigation system modified after Weaver's (1975) was employed. Six glass reservoirs each containing 26 liters of nitrogen-free nutrient solution (Broughton and Dilworth, 1971) and fitted with a constant head device (Marriott tube) were each connected to a surgical tubing main line (1.3 cm I.D., .32 cm wall). Stock nutrient solutions were diluted with tap water passed through an ultra violet sterilizer (Refco Products). The pH of the diluted nutrient solutions was adjusted to pH 7.3. Pressure heads in all six main lines were adjusted to 2.5 cm with the aid of calibrated sight



glasses.

Individual pots (1 liter plastic pots, Lab Tek Products) were connected to the main line via surgical tubing laterals (.95 cm I.D., .32 cm wall) and 14 ga. aluminum hypodermic needles (Monoject). Lateral lines were fitted to a hole punched in the bottom of the plastic pots such that the surgical tubing-pot connection was water tight. The other end of lateral lines were fitted to a hole punched in the bottom of the plastic pots such that the surgical tubing-pot connection was water tight. The other end of lateral lines were fitted to hypodermics which were pushed into the main line.

Pots were sterilized in a .6% sodium hypochlorite solution for 48 hours, rinsed with hot tap water, then rinsed with a 70% solution of ethanol.

Bottoms of pots were filled with sterile, coarse gravel (24 hours at 100°C) followed by sterile growth medium (autoclaved at 15 lbs. for 0.5 hours). The medium was composed of 50% washed vermiculite and 50% horticultural perlite (V:V). The pH of the medium was 7.2.

Four surface sterilized seeds (7 minutes with 3% sodium hypochlorite, rinsed in sterile .01 N HCl, followed by 10 sterile water rinses) were placed on the medium, covered with 2 cm additional medium followed by 1 cm of sterile aquarium gravel. Seeds of varieties Starr (bunch type) and Florida Giant (runner type) were obtained from the NIFTAL seed collection. Pots were connected to their assigned subirrigation system and free water levels adjusted to 2.5 cm.

Pots were thinned to two plants per pot 10 days after planting. Internal pot temperatures reached a maximum of 32°C during germination.

Pots receiving mineral nitrogen were given  $\text{NH}_4\text{NO}_3$  (3.56 M) according to the following schedule:

Days from Planting	Mg N/pot
14	50
20	200
29	100
36	100

The  $\text{NH}_4\text{NO}_3$  was placed 4-5 cm below the surface of each pot with a sterile Pasteur pipette.

Plants were inoculated ten days after planting; each plant receiving 1 ml of a turbid suspension of yeast extract mannitol broth (YMB) (Vincent, 1970) containing approximately  $10^8$  viable cells/ml of the appropriate strain of Rhizobium. Cultures were placed near the root system of the seedlings with a pipette.

Plant observations were made five times during the course of the experiment. Size relative to plus nitrogen treatments and leaf color were noted. Plants were observed daily for flower initiation beginning 28 days from planting.

#### Harvesting Procedure

Pots were harvested by block after 40 days of growth. Plants were cut just beneath the cotyledon. Roots were shaken free of medium and incubated for one hour in 4.7% acetylene in 2100 ml plastic bottles fitted with a serum stopper. A 10 ml gas sample was withdrawn with an evacuated tube (Vacuutainer). A .5 cc sample from the tube was later injected into a Varian Aereograph 940 gas chromatograph equipped with a column packed with Poropak R to detect the presence of ethylene

(Hardy, et al., 1968). Acetylene concentrations in blocks I and II, as determined by gas chromatography, were low (approximately 1.4% acetylene). There apparently was a gas leak in the acetylene storage bladder.

Shoots of all pots were dried at 65°C for 48 hours and weighed. Dried shoots were ground, and N determined by a modified Kjeldahl method using the colorimetric determination of ammonium (Mitchell, 1972).

Roots were washed clean of media and graded for nodule number, nodule diameter (average of 10 nodules per root system), the percent effective nodules (based on visual presence or absence of leghemoglobin) and the extent of tap root nodulation. These ratings were applied to a nodule grading system based on a maximum total value for efficient nodulation of 10 (Sandman, 1970). Roots with nodules were dried at 65°C for 48 hours and weighed.

### Statistical Design

Treatments were laid out in a randomized complete block design with each nitrogen source treatment assigned to its own subirrigation system. The four blocks were arranged along the length of a greenhouse bench. Uninoculated controls of both cultivars were connected to the subirrigation systems of the rhizobial treatments. In addition, four uninoculated pots of cultivar Florida Giant were connected to the sub-irrigation system provided for plus nitrogen treatments. One pot, removed from the experiment due to disease, was accounted for with a missing pot formula from Snedecor and Cochran (1974).

## RESULTS

There were significant differences among the shoot N of the various strain treatments as well as differences between the two cultivars (TABLE 1). Shoot dry weights for this experiment are in Appendix B. Strain TAL 236 was superior to TAL 309 on both cultivars. While TAL 309 gave higher shoot N yields than TAL 170 differences within each cultivar were not significant. The mean N yield of TAL 309 across both cultivars was, however, significantly greater than TAL 170. Dry weight differences between the effective strains TAL 309 and TAL 170 and ineffective strains were not significant even though total shoot N differences were significant. This was due to the late inoculation and early harvest of this experiment. Differences in total N were due to differences in shoot N concentrations.

A significant interaction between nitrogen source and cultivar treatment combinations can be readily seen. The yield of Florida Giant when inoculated with TAL 236 was not different than its mineral nitrogen treatment, but cultivar Starr, when inoculated with TAL 236, yielded only 61% of its respective mineral nitrogen treatment.

The large seeded cultivar, Florida Giant (1.2 g/seed) consistently yielded more N than Starr (.6 g/seed). The response of Starr to mineral nitrogen was 1.7 times that of its best rhizobial treatment. The yields of Florida Giant and Starr when given  $\text{NH}_4\text{NO}_3$  were similar; suggesting that both cultivars had equal genetic potential in early seedling growth. Cultivar Starr does not appear to have as high a symbiotic potential as Florida Giant with these

TABLE 1. -- SHOOT N OF TWO CULTIVARS OF PEANUT INOCULATED WITH FIVE STRAINS OF RHIZOBIUM SPP.

Strain	Florida Giant	Starr	Mean
	----- mg N/pot -----		
NH <sub>4</sub> NO <sub>3</sub>	386 a	323 a	355 a
TAL 236	410 a	197 b	303 b
TAL 309	286 b	153 c	220 c
TAL 170	248 b	149 c	190 d
TAL 423	80 c	53 d	67 e
Uninoculated	86 c	39 d	62 c
TAL 174	77 c	40 d	58 e
Mean	225 a	136 b	

Means within group not followed by the same letter differ at  $P \leq .05$  as given by Duncan's New Multiple Range Test.

strains. The mean N yield (314 mg N/pot) of effective strains TAL 236, TAL 309, TAL 170 on Florida Giant was equal to 82% of the mineral N treatment. The mean of the same effective strain treatments on Starr was 197 mg N, which is only 52% of Starr's mineral nitrogen yield.

Large within treatment variation for the acetylene reducing strains eliminated the possibility of distinguishing differences between the active strains (TABLE 2). However, the assay distinguishes between those strains which were capable of reducing acetylene (TAL 236, TAL 170, TAL 309) and the inactive strains (TAL 423, TAL 174).

Partitioning of the dry matter production revealed significant differences between rhizobial treatments and between the cultivars (TABLE 3). There was no interaction between N source and cultivars for the ratio of shoot weight to root weight (including nodules). Florida Giant had a significantly higher ( $P \leq .01$ ) ratio of shoot weight (4.8) than Starr (3.7). The mineral N and TAL 236 treatments tended to have higher shoot to root ratios than the less effective rhizobial treatments. The ratio of shoot weight to root weight when regressed on total dry matter production yielded a highly significant positive linear correlation ( $r = .91^{**}$ ). The same ratio regressed on shoot N was correlated at  $r = .74^{**}$ .

The nitrogen source had no effect on the number of days needed for 50% of the pots of a treatment to show flower initiation (Appendix C). Cultivar Starr did, however, flower significantly earlier than Florida Giant (33.7 days versus 35.6,  $P \leq .01$ ).

Estimates of nodule number could not be related to effectiveness

(Appendix D). The one nodule parameter that was related to effectiveness was the average diameter of nodules. Ineffective treatments (TAL 423, TAL 174) had nodules that averaged 1 mm or less in diameter as contrasted to effective treatments which had nodules with average diameters ranging from 1.3 mm to 2.1 mm. Florida Giant had larger nodules than Starr. Nodule numbers for the two cultivars were similar except in one treatment. TAL 174 consistently produced less than 100 nodules on Florida Giant whereas the same culture produced over 200 nodules on Starr. The average diameter of nodules formed by TAL 174 on Florida Giant was also consistently smaller than those on Starr. Growth could not be related to the nodule efficiency index of Sandman (1970).

Plant observations made throughout the experiment for leaf color and size indicated distinctly earlier greening of foliage for plants inoculated with TAL 236. This occurred at 32 days. Plants inoculated with TAL 309 and TAL 170 showed color changes five days later. Treatments TAL 174 and TAL 423 were yellow to yellow green in appearance. The uninoculated controls had foliage color similar to those of the ineffective rhizobial treatments.

The N yield of uninoculated pots of Florida Giant which were connected to the same subirrigation system as pots receiving mineral N was not different than other uninoculated Florida Giant pots which were connected to the system of rhizobial treatments. Control pots of Florida Giant connected to the plus N systems provided a mean yield of 71 mg N per pot. Controls connected to rhizobial treatment systems yielded 86 mg N per pot as seen in Table 1. The two means

TABLE 2. -- ACETYLENE REDUCTION FOR TWO CULTIVARS OF PEANUT INOCULATED  
WITH FIVE STRAINS OF RHIZOBIUM SPP.

Strain	Florida Giant	Starr	Mean
	----- uM C <sub>2</sub> H <sub>2</sub> /pot/hr -----		
TAL 170	21.5 a	14.5 a	17.7 a
TAL 309	14.5 a	17.1 a	15.8 a
TAL 236	16.5 a	14.9 a	15.7 a
TAL 174	0.0 b	0.0 b	0.0 b
TAL 423	0.0 b	0.0 b	0.0 b
NH <sub>4</sub> NO <sub>3</sub>	0.0 b	0.0 b	0.0 b
Uninoculated	0.0 b	0.0 b	0.0 b
Mean	17.2 a	15.5 a	

Means within a group not followed by the same letter differ at  $P \leq .05$   
as given by Duncan's New Multiple Range Test.



TABLE 3. -- THE RATIO OF SHOOT WEIGHT FOR TWO CULTIVARS OF PEANUT  
INOCULATED WITH FIVE STRAINS OF RHIZOBIUM SPP.

Strain	Florida Giant	Starr	Mean
	----- shoot wt/root wt -----		
NH <sub>4</sub> NO <sub>3</sub>	7.6 a	7.7 a	7.7 a
TAL 236	4.9 b	3.6 b	4.3 b
TAL 309	4.6 b	3.0 b	3.8 b
TAL 174	4.2 b	3.0 b	3.6 b
Uninoculated	4.3 b	2.7 b	3.8 b
TAL 170	3.8 b	3.1 b	3.5 b
TAL 423	4.0 b	2.8 b	3.4 b
Mean	4.8 a	3.7 b	

Means not followed by the same letter are significant at  $P \leq .01$  as given by Duncan's New Multiple Range Test.

are not different ( $P \leq .68$ ). Apparently, no significant amount of mineral N diffused into the sub-irrigation system.

None of the uninoculated pots for either Florida Giant or Starr reduced acetylene. Nodule observations of these pots, however, indicated that some contamination took place. No more than ten nodules were found on contaminated controls. The few nodules found on contaminated pots tended to be larger than those on inoculated pots.

### DISCUSSION

The promiscuous nodulating behavior of the peanut with cowpea type Rhizobium and the wide distribution of these Rhizobium have made it difficult to demonstrate a clear response to field inoculation. Failure to obtain a response with selected strains has occurred even when up to 60% of the nodules were formed by the inoculum strain (Diatloff and Langford, 1975; Van der Merwe, et al., 1974). Finding host genotypes that can fix large amounts of nitrogen relative to their potential with mineral nitrogen may be one approach to increasing nitrogen fixation with peanut. To test the hypothesis that genotypes of peanut vary in their symbiotic capacities it is necessary to use strains of Rhizobium that are highly effective on more than one variety.

Strains TAL 236, TAL 170, and TAL 309 (CB756) have been previously shown to be highly effective on peanut cultivar Burpee Spanish at NifTAL. TAL 309 (CB756) is a widely used, effective, broad spectrum cowpea type Rhizobium.

Experiment 1 showed that the two cultivars' potentials for growth with mineral nitrogen were more similar than their growth when the source of N is the symbiosis. The fact that cultivar Starr's seed size was only half that of Florida Giant's did not appear to seriously reduce its early growth potential with mineral nitrogen.

Early vigor of the two cultivars when given mineral nitrogen did not appear to be related to their symbiotic capabilities. Gibson (1962) showed that the early N yield of 15 alfalfa (Medicago sativa) varieties given  $\text{NH}_4\text{NO}_3$  were more uniform than the varieties' mean N yields when in symbiosis with six effective strains of Rhizobium meliloti. Mean symbiotic cultivar yields across the six strains varied more than the mean rhizobial treatments across the 15 cultivars. Although this experiment with peanut involved only two cultivars and three previously tested, effective strains of Rhizobium, the difference between cultivar yields with the symbiosis varied more than the inoculant treatments.

No serious strain by host interactions were revealed. All three effective strains reduced acetylene on both cultivars. The ranking of the strains on the two cultivars was the same; TAL 236 was superior on both cultivars. The ineffective strains behaved similarly on both cultivars; they did not reduce acetylene on either cultivar nor did the cultivar means differ from the uninoculated pots.

Since the two peanut cultivars have a similar early yield potential with mineral N and yet differ so greatly with respect to their symbiotic yields with three previously selected effective strains, it seems reasonable to conclude that the symbiotic capacities of the two

cultivars differ considerably. The symbiotic potential of these two cultivars is independent of potential with mineral N. The fact that the rank of the three strains is the same on both cultivars lends support to this hypothesis. Starr consistently yielded less with each effective strain than Florida Giant.

The partitioning of dry matter in plants has been shown to be a function of mineral nutrition. Brower (1962) demonstrated that the ratio of shoot weight to root weight of corn had a positive relationship with the amount of  $\text{NO}_3$  supplied. In this experiment, peanut showed similar trends. The shoot to root ratio was highest and nearly identical for both cultivars when given mineral N. Florida Giant consistently had a higher shoot to root ratio than Starr for both effective and ineffective strain treatments. This morphological characteristic of Florida Giant appears to be related to its higher symbiotic shoot N yield. Thus, improved N nutrition either from symbiotic or mineral sources reduces the partitioning of dry matter to the roots of peanut and thereby provides for greater shoot growth.

Neither mode of N supply nor degree of rhizobial effectiveness affected the number of days to floral initiation. Hardy and Havelka (1976) have shown that peak nitrogenase activities take place in the early stages of flowering. If the degree of effectiveness altered the time to flower initiation this could in turn have a feedback effect on nitrogen fixation. Starr flowered significantly earlier than Florida Giant. The earlier physiological change did not result in greater nitrogen accumulation for cultivar Starr.

Nodule gradings according to the system of Sandman (1970) could

not be correlated with N accumulation. One nodule parameter that did correlate with nitrogen fixation was nodule diameter ( $r = .78^{**}$ ). Both cultivars had approximately the same number of nodules with the effective Rhizobium. The average nodule diameter for the effective Rhizobium on Florida Giant was significantly greater than Starr ( $t = 4.4^{***}$ , 22 d.f.). Since average nodule numbers were the same and the average nodule diameter was greater for Florida Giant it implies that there was more active nodule tissue on the effective Florida Giant treatments than that found on Starr. Average nodule diameter appears to be a good indicator of nitrogen fixation when number of nodules are constant.

The observation that ineffective strain TAL 174 consistently produced fewer and smaller nodules on Florida Giant than on Starr implies that Florida Giant may possess partial resistance to infection by this strain.

The most efficient strain, TAL 236, caused dark green foliage to appear 3-5 days prior to plants inoculated with the other effective strains, TAL 309 and TAL 170. Cultivar Florida Giant did not show dark green foliage earlier than Starr. Whether actual reduction of di-nitrogen occurred earlier in the higher yielding cultivar cannot be determined.

Shoot N of the plus N treatments (450 mg N added) indicated that over 75% of the applied N was recovered in the shoots of Florida Giant and 65% in Starr. These figures account for seed N assuming 4.8% N in the seeds (Smartt, 1976).

Uninoculated pots connected to the plus N irrigation system did

not differ from uninoculated pots connected to random irrigation systems having no plus nitrogen pots attached. Apparently, no significant amount of mineral N diffused into the system from pots receiving  $\text{NH}_4\text{NO}_3$ .

In conclusion, the growth system tested in this experiment appears to have a large growth capacity and is capable of providing adequate if incomplete control of contamination by extraneous Rhizobium. Having up to eight pots receiving 450 mg of mineral N each does not result in significant diffusion of N into the irrigation systems.

Despite large differences in seed size, early yield potentials of Florida Giant and Starr appear to be more similar when given  $\text{NH}_4\text{NO}_3$  than when relying on symbiosis with three strains of Rhizobium shown previously to be effective with peanut. Florida Giant is capable of fixing considerably more N in early growth than Starr with the three effective strains. This greater symbiotic capacity was related to larger shoot to root ratios. One strain, TAL 236, is superior to the other strains with both cultivars. Having shown no serious interactions between the cultivars and other effective strains, TAL 236 is the logical choice for testing additional cultivars of peanut.

Since the mean N yield of the cultivars across the effective strains varied more than the mean strain yields, selection of hosts for high symbiotic potential may be the most feasible way to increase N yields of peanuts.

## CHAPTER II

### EXPERIMENT 2: THE SYMBIOTIC EFFECTIVENESS OF SEVEN STRAINS OF RHIZOBIUM SPP. WITH TWO CULTIVARS OF PEANUT (ARACHIS HYPOGEA (L.))

#### ABSTRACT

Two cultivars of peanut (Arachis hypogea (L.)) were tested in the greenhouse for symbiotic effectiveness with seven strains of Rhizobium spp. Significant differences in rhizobial strains and significant strain by cultivar interactions were observed at the 45 day harvest. Strain TAL 1000 did not differ from the mineral nitrogen control on either cultivar and there was no strain by cultivar interaction. A Hawaiian isolate, TAL 1000, produced a mean shoot yield across both cultivars that was significantly greater than T-1, a standard Texas peanut strain.

The significant strain by cultivar interactions with two other effective strains demonstrated the danger in selecting superior strains of rhizobia on a single genotype. In one case the symbiosis of AH8 with cultivar Burpee Spanish would be classified as being superior. Yet the mean shoot yield of AH8 across both cultivars show AH8 to be significantly less efficient than TAL 1000.

An ineffective mutant of a normally effective strain (SU) was ineffective on both cultivars and therefore, not cultivar specific. An isolate of Acacia koa nodulated both cultivars but the symbiosis with this isolate (TAL 301) was ineffective.

The two cultivars had the same early yield potential with the

four effective strains as when given mineral nitrogen.

### INTRODUCTION

Screening for rhizobial effectiveness on peanut has indicated that the peanut, which is promiscuous in nodulating behavior also has a somewhat specific requirement for effectiveness (Dadarwal, 1974; Gaur, et al., 1974).

This experiment was conducted to identify additional superior strains of Rhizobium that are highly effective on two cultivars of peanut. Also, isolates made from peanut in Hawaii were compared with a highly effective strain from Texas.

An ineffective mutant of a Texas strain was also included to determine whether or not the ineffective trait is cultivar specific. The ineffective strain had been previously tested on another cultivar (Weaver, personal communication, Texas A & M University). Nodulation characteristics of the ineffective mutant were observed in order to compare these characteristics with other ineffective strains.

Habish and Khairi (1968) reported that isolates from Acacia spp. did not nodulate Arachis hypogea. This contradicts the findings of Gaur, et al. (1974) who showed Arachis hypogea to be extremely promiscuous in its nodulating behavior. An isolate from Acacia koa was therefore included in this test to help clarify the contradiction between the results of Gaur, et al. (1974) and Habish and Khairi (1968).

Results from Experiment 1 indicated that a strain superior on two cultivars could be identified. The objective of this experiment



was to identify another strain which is both highly effective and yet exhibits no interactions with two different cultivars. These broad spectrum strains will then be used to test additional cultivars of peanut for their symbiotic capacity in order to minimize the probability that poor cultivar performance in symbiotic capacity is due to low Rhizobium strain effectiveness.

## MATERIALS AND METHODS

### Growth System and Plant Culture

A subirrigation system modified after Weaver's (1975) and described in detail in Experiment 1 was employed. Pot media consisted of 60% perlite and 40% vermiculite (V:V) as opposed to the 50:50 ratio used in Experiment 1. Pots were planted with four surface sterilized seeds of one of two cultivars, Florunner (runner type) and Burpee Spanish (bunch type) as per Experiment 1. Each seed was inoculated at planting with .5 ml of one of seven rhizobial strain YMB cultures (Vincent, 1970).

An  $\text{NH}_4\text{NO}_3$  standard was employed for each cultivar. Applications of 450 mg N as  $\text{NH}_4\text{NO}_3$  was performed as in Experiment 1 according to the following schedule:

Days After Planting	Mg N Applied
13	50
24	100
26	100
35	100
40	100

Pots were thinned to two plants per pot 16 days after planting.

### Rhizobium Culture

Cultures of seven strains of Rhizobium spp. were grown in 25 ml of YMB (Vincent, 1970) for 8 days. The strains, described in Appendix F contained in excess of  $3 \times 10^8$  cells/ml as determined by plate count (Vincent, 1970).

### Harvest Procedure

Pots were harvested by block 45 days after planting. Shoots were cut beneath the cotyledon taking care to include all pegs of the plants. Shoots were dried at 65°C.

### Statistical Design

The design employed was a completely randomized block design. Blocks (4) consisted of a subirrigation system with each of the 18 treatment combinations (2 cultivars x 7 strains and plus and minus N) represented once in a block. Pots were randomized spatially on the greenhouse bench.

## RESULTS

There were no differences in shoot dry weight between the two cultivars but there were significant differences between rhizobial strains and significant strain by cultivar interactions (TABLE 4).

Strain TAL 1000 performed better on both cultivars than the standard Texas strain T-1. Strain AH8 was not different from TAL 1000 on cultivar Burpee Spanish but yielded significantly less than TAL 1000 on Florunner. Strain T-1 yielded significantly more than AH8 on Florunner but significantly less than AH8 on Burpee Spanish.

TABLE 4. -- SHOOT WEIGHTS OF TWO PEANUT CULTIVARS INOCULATED WITH SEVEN STRAINS OF RHIZOBIUM SPP.

Treatment	Burpee Spanish	Florunner	Mean
	----- g/pot -----		
450 mg N	14.1 a	14.7 a	14.4 a
TAL 1000	13.5 a	14.0 a	13.7 a
T-1	11.2 b	12.8 a	12.0 b
AH8	13.6 a	10.2 b	11.9 b
AH10	7.7 c	8.6 bc	8.2 c
SU-in	6.0 cd	6.8 cd	6.4 d
TAL301	5.4 d	6.7 cd	6.0 d
CONTROL	5.3 d	6.6 cd	6.0 d
UMKL 44	5.8 cd	5.3 d	5.4 d
Mean	9.2 a	9.5 a	

Means in the same group not followed by the same letter are significant at  $P \leq .05$  as given by Duncan's New Multiple Range Test.

In neither cultivar was the  $\text{NH}_4\text{NO}_3$  treatment better than TAL 1000. Strain means show that TAL 1000 was superior, being significantly better than any of the other effective strains when data from both cultivars were considered.

Strain AH10 was only partially effective, yielding more than the uninoculated control on both cultivars but only 59% of the yield obtained with TAL 1000.

Strain SU-ineff, a mutant of a normally effective strain (Weaver, 1975) was ineffective on both cultivars.

Plants inoculated with TAL 301, an isolate from Acacia koa, formed nodules but was ineffective on both cultivars.

The mean yields of Florunner and Burpee Spanish across all treatments were the same. The average yields for the two cultivars were nearly identical when given  $\text{NH}_4\text{NO}_3$  or when inoculated with the effective strains TAL 1000, T-1, or AH8 (12.8 g for Burpee Spanish, 12.5 g for Florunner).

Nodule observations after harvest showed that strain TAL 301 produced many small nodules compared to effective strains such as TAL 1000. The ineffective mutant, Su-ineff, produced nodules of a more normal size on Florunner but very small nodules on Burpee Spanish.

Contamination of uninoculated controls was observed. This contaminant nodulation was located on lateral roots rather than the tap root and did not appear to affect yields.

## DISCUSSION

This test showed that a strain could be selected which was superior on two cultivars. TAL 1000 proved to be superior on two distinctly different cultivars with very little interaction between the two cultivars and other strains. Therefore, TAL 1000 is a suitable strain for use in future examination of additional peanut cultivars. TAL 1000, AH8, and AH10 were isolated from Virginia runner peanut at the NifTAL site. At the time of isolation visual examination of nodules indicated that TAL 1000 appeared to be a highly effective strain. The amount of leghemoglobin was so great the nodule appeared to be red from the outside.

Plant observations during the experiment indicated that dark green foliage was present on plants inoculated with TAL 1000 four days earlier than those inoculated with strain T-1 and AH8. TAL 1000 caused foliage color changes a full eleven days before those inoculated with AH10. Greenhouse strain selection for symbiotic effectiveness may be selecting for early nodule formation or early activation of the nitrogenase enzymes system. Whether early performance of host strain combinations is indicative of their final field performance is not clear. Early initiation of nitrogen fixation would be a clear advantage in those field situations where soil nitrogen was at extremely low levels.

The highly significant cultivar by strain interactions between effective strains T-1 and AH8 indicate the danger of selecting for superior strains on only one genotype. Mean yields of AH8 and T-1 treatments gave the same yield and should be considered of equal

effectiveness. However, if strain selection with these two strains were to be undertaken with one cultivar or the other, one of the strains would be classified as a lesser effective strain. Furthermore, if single cultivar strain selection with these strains would have been undertaken on Burpee Spanish, AH8 would have been classified as a highly effective strain. However, it is effective only for that cultivar; AH8 is significantly less effective than TAL 1000 when data from both cultivars are considered.

Strain SU-ineff, an ineffective mutant of a normally effective strain, was ineffective on both cultivars. This shows that the trait was not cultivar specific. Nodules produced by SU-ineff were smaller than nodules produced by the effective strains but larger than those incited by other ineffective strains.

TAL 301, an isolate from Acacia koa did form numerous but very small nodules on both cultivars. This confirms the observations made by Gaur (1974) as opposed to statements made by Habish and Khairi (1968). Isolates from at least some Acacia spp. are therefore, capable of nodule formation with peanut.

In conclusion, data from Experiments 1 and 2 indicate that testing of rhizobia with a single cultivar may lead to inappropriate selection of strains for superior effectiveness. Some strains, while superior with an individual genotype, are inferior when considered with two genotypes. Single cultivar tests for general strain effectiveness do appear to be valid for identifying those strains which are effective on peanut as opposed to being completely ineffective. No strain in either Experiments 1 and 2 was found to be effective on

one genotype and completely ineffective on another. The significant strain by cultivar interactions indicate that strains used for commercial inocula should be tested on the specific cultivars which are to be planted.

It does appear that strains which are superior on at least two different cultivars can be identified. TAL 236 has been shown to be superior relative to some other known effective strains. TAL 236 was tested on a total of three cultivars. TAL 1000 was shown to be significantly better than some other peanut strains on two cultivars.

Some cultivars such as Burpee Spanish and Florunner have similar early yield potentials with a variety of effective strains and mineral nitrogen. Other cultivars, as shown in Experiment 1, seem to differ in their symbiotic potential with strains selected previously for effectiveness on peanut.

## CHAPTER III

### EXPERIMENT 3: TIME TO NODULE FORMATION OF THREE CULTIVARS OF PEANUT (ARACHIS HYPOGEA (L.)) INOCULATED WITH TEN STRAINS OF RHIZOBIUM SPP.

#### ABSTRACT

A growth room study was undertaken to determine if three cultivars of peanut (Arachis hypogea (L.)) and ten strains of invasive Rhizobium spp. varied in the time to nodule formation (TNF).

Significant differences were found for TNF among the three cultivars. TNF of the cultivars was positively correlated with seed size.

Strain differences in TNF and strain by cultivar interactions were observed. TNF could not, however, always be related to symbiotic efficiency as determined by Experiments 1 and 2. The implication of this study is that although the time to activation of the nitrogenase system may be an important determinant of symbiotic performance, this is not necessarily well correlated with time to nodule formation.

#### INTRODUCTION

Results of plant observations in Experiments 1 and 2 indicated that some rhizobial strain-cultivar combinations seemed to initiate nitrogen fixation earlier than other combinations. In Experiment 1, plants inoculated with strain TAL 236 exhibited dark green foliage five days before plants inoculated with less effective strains such



as TAL 309 and TAL 170. Similarly, periodic plant observations in Experiment 2 indicated that plants receiving strain TAL 1000 had distinct changes in foliage color four days prior to the next best strain, T-1. Strain AH8 also showed color differences three days prior to changes in plants treated with partially effective strain AH10. Strain AH8 was significantly better than AH10 for dry matter yield. These data suggested that symbiotic efficiency as revealed in short greenhouse pot tests was a function of the speed at which nitrogen fixation is initiated. Whether the time to the onset of nitrogen fixation was related to the time to nodule formation (TNF) is not clear.

Nutman (1967) showed that TNF was both host and strain dependent for Trifolium subterraneum. Early nodulation was found to be polygenetically inherited in a complex fashion. Nutman did not demonstrate a significant interaction between the host and strain for TNF. Variability in TNF for Trifolium ambiguum (Hely, 1957), Trifolium pratense (Nutman, 1946) and Trifolium repens (Jones, 1963) was larger than the variability found in Trifolium subterraneum (Nutman, 1967). Robinson (1969) found that differences in TNF between species of Trifolium and Rhizobium trifolii isolates were related to symbiotic efficiency. The least efficient host-strain combinations were the slowest to nodulate.

This experiment was conducted to determine whether variability in TNF exists for rhizobial strains infecting different genotypes of peanut and to determine whether TNF is related to the symbiotic effectiveness of the host-strain combinations as determined in Experi-

ments 1 and 2.

#### MATERIALS AND METHODS

Surface sterilized seed (8 minutes in 3% sodium hypochlorite, sterile .01 N HCl rinse, 4 sterile water rinses) of cultivars Florida Giant, Florunner, and Burpee Spanish were planted in sterile plastic seedling growth pouches (Scientific Products) according to Weaver (1972). The radicle ends of 3 seeds were placed in separate holes punched in the bottom of the paper planting trough. Pouches were filled with 30 ml of sterile nitrogen-free nutrient solution (Broughton and Dilworth, 1971). Pouches were alternately replenished with nutrient solution and de-ionized water.

Pouches were thinned to two plants per pouch six days after planting. Emerging radicles of remaining seedlings were inoculated with .5 ml of the appropriate YMB culture. All cultures had at least  $1 \times 10^9$  cells per ml. Pouches were suspended on racks in a growth room.

One week after inoculation, daily observations of root systems were made to detect the presence of nodules. Plants in some pouches did not grow well and were removed from the experiment before observations began, creating treatments of unequal sample size. Analysis of data was, therefore, carried out according to methods outlined by Snedecor and Cochran (1974). Sixteen sterile uninoculated controls were included in the experiment.

## RESULTS

Significant differences between the cultivars, rhizobial treatments, and strain by cultivar interactions for TNF were apparent (TABLE 5). The large seeded runner variety, Florida Giant (1.2 g/seed), was significantly slower to nodulate than either Florunner (.77 g/seed) and Burpee Spanish (.52 g/seed). Florunner was slower to form nodules than Burpee Spanish. Florida Giant required, on average, 20.1 days to nodulate versus 16.3 for Florunner and 14.5 for Burpee Spanish.

Significant differences in TNF for rhizobial treatments were also found. Effective strains TAL 1000 and AH8 had lower TNF than the ineffective mutant, SU-ineff. The relationship between TNF and efficient symbiotic capability is, however, not consistent. Strain SU-ineff has a significantly lower TNF than effective strains TAL 309, TAL 170, AH10, and TAL 236. Ineffective strains TAL 423 and UMKL 44 were the slowest strains to form nodules.

## DISCUSSION

Differences between effective strains in their nitrogen fixing efficiency were shown in Experiments 1 and 2. Figures for TNF in this experiment indicated that TAL 236 had a longer TNF than less efficient strains TAL 309 and 170. Similarly, AH8 had a significantly shorter TNF than T-1 yet the two strains are comparable in nitrogen fixation efficiency.

It appears then, that TNF is both host and rhizobial strain

TABLE 5. -- TIME TO NODULE FORMATION (TNF) FOR THREE CULTIVARS OF  
PEANUT INOCULATED WITH 10 STRAINS OF RHIZOBIUM SPP.

Strain	Burpee Spanish	Florunner	Florida Giant	Mean
	----- days -----			
AH8	11.5	12.4	16.8	13.3 a
TAL 1000	13.2	13.0	15.0	13.5 ab
SU-ineff	13.0	19.5	18.0	15.0 bc
T-1	15.0	14.3	17.7	16.5 cd
TAL 309	14.0	14.5	21.4	17.2 d
TAL 170	15.2	15.2	22.2	17.5 d
AH10	13.7	12.5	20.9	17.7 d
TAL 236	17.3	17.3	19.8	18.4 de
UMKL 44	17.4	19.6	24.5	20.1 ef
TAL 423	19.0	20.6	23.8	21.7 f
Mean	14.5 a	16.3 b	20.1 c	

Means not followed by same letter differ at  $P \leq .05$ .

related. Larger seeded hosts tend to nodulate slower than smaller seeded types. The TNF figures for the cultivars vary directly with seed size. Greater seed nitrogen reserves probably inhibit nodule initiation until nitrogen is depleted to some critical level in the plant.

TNF was not related to rhizobial strain growth rate on agar or broth media. Colony size on YMA (Vincent, 1970) impregnated with bromthymol blue and turbidity in YMB show UMKL 44 to be faster growing than AH8 and AH3. UMKL 44 had a larger TNF than the other two strains. Observations of broth cultures showed that T-1 grew faster than AH8 and AH3 yet took longer to form nodules.

Whether TNF in this growth room study correlates exactly with TNF in the greenhouse pots used for effectiveness tests cannot be determined. If the correlation is close this study would indicate that the time to the activation of the nitrogenase enzyme system would be the critical determinant in early rhizobial strain effectiveness rather than the initiation of nodules, and that these two factors are necessarily well correlated.

## CHAPTER IV

### EXPERIMENT 4: GREENHOUSE STUDIES ON THE SYMBIOTIC EFFECTIVENESS OF TWELVE TROPICAL PEANUT CULTIVARS

#### ABSTRACT

A greenhouse experiment was conducted to compare the early nitrogen fixing capacities of 12 cultivars of peanut (Arachis hypogea (L.)). One liter plastic pots containing vermiculite and perlite and connected to a subirrigation system were used to test the effect of inoculation with three strains of Rhizobium spp. in comparison with an  $\text{NH}_4\text{NO}_3$  control on each cultivar. All three strains of Rhizobium had previously been shown to be highly effective with at least two cultivars of peanut. Dry weight data from five week old plants indicated that cultivar yields were relatively more variable when relying on the symbiosis than when given  $\text{NH}_4\text{NO}_3$ . Cultivar means composed of the three Rhizobium treatments (symbiotic mean) ranged from 6.3 g/pot to 12.5 g/pot. Means of the same cultivars given mineral N ranged from 10.6 g/pot to 15.8 g/pot. The symbiotic means of cultivars relative to their respective yield with  $\text{NH}_4\text{NO}_3$  ranged from .47 to .87. Data for total shoot N showed a similar trend. Significant cultivar by Rhizobium strain interactions were also found. The existence of strain by cultivar interactions indicates the need to reconsider the selection of strains of Rhizobium on a single cultivar.

## INTRODUCTION

As stated previously in Experiment 1, the peanut is the most important legume in the tropics. It is of significant importance to small farmers in the tropics as both a pulse and a cash crop. Despite the importance of this crop little research has been conducted concerning the peanut-Rhizobium symbiosis.

One factor that may have limited research on this symbiosis is the fact that the peanut is one of the most promiscuously nodulating legumes (Gaur, et al., 1974; Dart, 1974). A wide range of slow growing, cowpea type Rhizobium nodulate peanut. Experiment 2 showed that isolates from such diverse origins as Acacia koa and Calipogonium muconoides nodulated peanut. The pervasive nature of these types of organisms has made it difficult to demonstrate a clear response to inoculation (Van der Merwe, et al., 1974; Diatloff and Langford, 1975; Subba Rao, 1976). Whether the failure to demonstrate an inoculation response is the result of poor inoculum competitiveness, high effectiveness of native strains, or the presence of some other yield limiting factor is not always clear. However, Diatloff and Langford (1975) did have 50% of the nodules of field grown peanut formed by the inoculum strain (TAL 309/CB756) and showed no response. They found that some soil strains were equally as effective as their inoculum in pure culture tests.

One approach to increasing the symbiotic N yield of peanut may be to develop plant genotypes that have a high symbiotic capacity with a number of rhizobial strains. Gibson (1962) showed that when fifteen cultivars of Medicago sativa were grown in combination with six effec-

tive strains of Rhizobium meliloti average cultivar yields varied more than average strain yields even though the response of the cultivars to  $\text{NH}_4\text{NO}_3$  was almost identical. High fixing varieties yielded 82% of their  $\text{NH}_4\text{NO}_3$  controls. Low fixing types yielded only 33% of their mineral N treatment. The lowest mean strain yield, on the other hand, was 71% of the highest strain mean. Gibson's data showed a highly significant variety by strain interaction. Crosses of low fixing varieties by high fixing varieties indicated that symbiotic capacity could be inherited. Large within variety variation for symbiotic capacity was also found. This variation was greater for genotypes in the intermediate range of symbiotic capacity.

Other workers have noted host varietal differences in nitrogen fixation or nodulation characteristics on Glycine max (Johnson and Means, 1960) and Trifolium spp. (Nutman, 1961; Gibson, 1964). Workers in Minnesota reported to have doubled the nitrogen fixation capacities of some Medicago sativa lines through selection (Seetin and Barnes, 1977). Duhigg, et al. (1978) found significant variation for nitrogen fixation within the cultivated alfalfa variety 'Mesilla.'

A number of cultivated genotypes of peanut, having commercial potential in the tropics were examined in this experiment in order to identify varieties which have a high capacity for nitrogen fixation in early growth. A large application of  $\text{NH}_4\text{NO}_3$  was administered to each cultivar as a standard of genetic potential. Comparison of symbiotic yields to a nitrogen standard should reduce the apparent effects that general vigor and seed size play in early nitrogen fixation. Each cultivar was tested with three highly effective



strains of Rhizobium. The strains, TAL 1000, TAL 236, and TAL 309 (CB756) were all chosen on the basis of prior effectiveness tests. Each strain had been proven to be highly effective on at least two cultivars and did not show significant strain by cultivar interactions on the cultivars tested. TAL 236 has been tested with three cultivars at NifTAL, TAL 309 was tested with three cultivars at NifTAL and others at different research centers. TAL 1000 was tested on two cultivars. By prior selection of strains on multiple host varieties it is probable that more broad spectrum strains have been selected. This should reduce the likelihood that low cultivar yields will be strictly strain induced.

This experiment was, therefore, undertaken to determine whether variation in symbiotic capacity relative to genetic potential exists among cultivars of peanut. Another objective of this experiment was to repeat the earlier procedure of selecting broad spectrum highly effective strains using only two or three cultivars.

## MATERIALS AND METHODS

### Germplasm

Seeds of 12 peanut cultivars identified as having significant potential in the tropics were provided by D. E. McCloud of the International Peanut Program, Gainesville, Florida. Oven dry weights were taken to determine what, if any, effect seed weight had on final shoot yield.

## Growth System and Plant Culture

A modified subirrigation system after Weaver (1975) was used. The physical arrangement of this system has been described in detail in Experiment 1.

The bottom 1 cm of one liter plastic pots, sterilized in 1% NaOCl for 24 hours, were filled with coarse, sterilized gravel, then filled to the top with a sterile mixture of perlite (grade II) and expanded vermiculite 1:1 (V:V). Seeds were surface sterilized in 1% NaOCl for 3 minutes, followed by eight sterile water rinses, and planted four to a pot (radicle end down) at a depth of 2 cm after inoculation of the pot. Sterile aquarium gravel was placed over the surface of the media. Pots were moved to the greenhouse at day 4 and connected to the sub-irrigation system.

Free water levels in the system were maintained at approximately 2.1 cm with the aid of previously calibrated sight glasses.

Stock solutions of a nitrogen-free nutrient solution (Broughton and Dilworth, 1971) were pipetted into calibrated glass reservoirs filled with tap water passed through an ultraviolet sterilizer (Refco Products). Nitrogen was delivered to plus N treatments in the form of  $\text{NH}_4\text{NO}_3$  added to the appropriate reservoir. The concentration of N in the reservoir was adjusted according to the following schedule:

Days from Planting	ppm N in solution
4 - 18	50
19 - 25	100
26 - 32	150
33 - 43	200

The pH of both N-free and plus N nutrient solutions was adjusted to 7.6. The pH of the rooting media at harvest was 5.6.

Pot experiments reached 38°C in late afternoon during germination. To avoid adverse effects of this extreme temperature, a shade cloth (50% transmission) was placed over the greenhouse benches at day 5 and removed at day 10. Pot temperatures reached a maximum of 33°C after a plant canopy was established.

Inoculum cultures were YMB. Determination of viable counts prior to inoculation was by a standard spread-plate method (Vincent, 1970). Culture broth was pipetted into a 2 cm hole in the media where the seed was to be placed.

#### Harvest Procedure

Forty three day old plants were harvested, one block per day, at 11:00 a.m. The acetylene reduction assay was performed in 10% acetylene as described in Experiment 1. Shoots were cut below the cotyledon, and dried at 60°C. Shoots were then ground, digested, and ammonium determined after Mitchell (1972).

Nodulation was observed and rated for the following characteristics: percent effective; nodule number; nodule diameter; and intensity of tap root nodulation. The methodology of these observations followed that of Sandman (1970).

#### Statistical Design

A modified split plot design was employed (Snedecor and Cochran, 1974) with the main plots consisting of individual reservoir main-line systems. Three bacterial and a plus nitrogen treatment were each assigned to individual reservoir systems. Cultivars comprised the subplots and all treatment combinations were replicated 3 times in a

randomized block design. This design provided for greater precision in measuring cultivar differences and cultivar by Rhizobium treatment interactions. Since block effects are confounded with main plot effects so differences between strains could not be statistically measured. The inability to statistically distinguish between N-source treatments was not considered critical since all three strains of Rhizobium had previously been shown to be effective on peanut and the identification of superior strains was therefore not an objective of this experiment. However, an approximation of symbiotic performance with each strain was evaluated by comparing the dry weight of cultivar-Rhizobium treatments to the cultivar's corresponding dry weight in the presence of mineral nitrogen.

## RESULTS

There were significant shoot dry weight differences between cultivars (TABLE 6). Significant strain by cultivar interactions were also present. Cultivar means (the mean of all treatments including  $\text{NH}_4\text{NO}_3$ ) could be separated into three groups. Early Bunch and Giza-4 are significantly higher yielding than the other cultivars. Most cultivars fell within a large intermediate group that had shoot yields in the range of 9.0 g/pot to 10.4 g/pot. Two cultivars performed poorly; Makula Red and F3-261. Cultivar F3-261 did not appear to be adapted to the growth system used in this experiment, since it yielded poorly even when provided with mineral N. The cultivars also separated into similar groups when the cultivar means of symbiotic treatments (mean excluding N treatment) only are consid-

TABLE 6. -- SHOOT DRY WEIGHT OF TWELVE CULTIVARS OF PEANUT INOCULATED WITH THREE STRAINS OF RHIZOBIUM SPP.

CULTIVAR	AMMONIUM NITRATE	INOCULATION TREATMENT ( <u>Rhizobium</u> strains)						Cultivar Mean
		TAL 1000		TAL 236		TAL 309		
		Dry Weight	% of N Control	Dry Weight	% of N Control	Dry Weight	% of N Control	
	g/pot	g/pot	%	g/pot	%	g/pot	%	g/pot
Early Bunch	14.4	16.9	117	14.3	99	6.3	44	13.0 a
Giza-4	15.1	17.8	118	8.0	53	11.0	73	13.0 a
Tato-34	14.8	13.5	91	8.9	60	4.5	30	10.4 b
Bachimba	14.6	9.5	65	8.3	57	8.4	58	10.2 b
Valencia R-2	11.2	16.2	145	8.0	71	4.7	42	10.0 b
Col. Correntino	12.0	8.3	69	9.0	75	8.9	74	9.6 bc
156 of Cuba	15.8	8.9	56	3.6	23	9.9	63	9.6 bc
Sulasmith	12.9	13.8	107	6.2	48	4.7	46	9.4 bc
Blanco Rio Seg.	10.6	9.6	91	6.5	61	9.8	92	9.1 bc
Egret	10.8	9.8	91	8.4	78	7.0	65	9.0 bc
Makula Red	12.1	9.3	77	5.3	44	4.4	36	7.8 cd
F3-261	6.6	7.0	106	4.6	68	5.8	88	6.0 d

<u>Source</u>	<u>d.f.</u>	<u>F</u>	
Cultivar	11	9.2**	LSD.05 for individual treatment combinations = 3.6
Cultivar x strain	35	4.0**	LSD.05 for cultivar grand mean = 1.8

ered. Again, Early Bunch and Giza-4 are significantly better than the other cultivars. Makula Red and F3-261 gave significantly lower yields. One cultivar, 156 of Cuba, had a very low symbiotic yield which was not significantly different than Makula Red or F3-261.

The yields of all cultivars, except F3-261, were fairly uniform when given  $\text{NH}_4\text{NO}_3$ . If F3-261 is ignored, the lowest yield with mineral N (10.6 g/pot for Blanco Rio Seg) was 70% of the highest yielding cultivar given  $\text{NH}_4\text{NO}_3$  (15.8 g/pot for 156 of Cuba). The yield range of symbiotic treatments was much greater. 156 of Cuba yielded only 3.6 g with TAL 236 while Giza-4 yielded 17.8 g with TAL 1000.

TABLE 7 shows similar cultivar results for total shoot N. While a dry weight response to mineral N on almost all cultivars was obtained, the figures for total shoot N showed that TAL 1000 generally produced more total N than the application of  $\text{NH}_4\text{NO}_3$ . The concentration of N in the shoots of plants given mineral N was lower than most symbiotic treatments. The pattern of total shoot N was similar to that for shoot dry weight. Giza-4 and Early Bunch were significantly greater than the others. Makula Red and F3-261 accumulated significantly less N than most cultivars. The large intermediate group did not always fall into the same ranking pattern as they did for shoot dry weight.

Data for acetylene reduction is included in Appendix E. There was no correlation between dry weight and acetylene reduction. The highest yielding cultivars, Early Bunch for example, had the lowest acetylene reduction values. This indicates that some factor other

than nitrogen was limiting growth and photosynthate supply at the time of harvest.

#### DISCUSSION

Peanut genotypes may vary in their early symbiotic capacity with a variety of known effective strains of Rhizobium. Results from Experiment 1 demonstrated this possibility and the present experiment offers additional evidence.

Symbiotic efficiency is a function of the host genotype, rhizobial strain, and the environment. Under the environmental conditions defined for this experiment and with this group of Rhizobium, the cultivars varied considerably in their early symbiotic capacity. This variation was significant in terms of absolute differences and in terms of the cultivars' symbiotic yields relative to their respective yields with mineral N. The range of cultivar yields with mineral N was considerably more narrow than the range of cultivar yields when N supply was from symbiosis. This indicates that differences in general genetic plant vigor were not the sole determinants of symbiotic capacity with this group of Rhizobium strains. This observation is in agreement with that made by Gibson (1962) on Medicago sativa.

Although the three strains of Rhizobium utilized in Experiment 4 had been previously selected for high efficiency and lack of interaction with two or more cultivars (runner and bunch type), significant strain by cultivar interactions were revealed in this study. For example, yields for TAL 236 were greater than TAL 309 on Early Bunch. Yields with these strains were reversed on the other high yielding

TABLE 7. -- SHOOT N OF TWELVE CULTIVARS OF PEANUT INOCULATED WITH  
THREE STRAINS OF RHIZOBIUM SPP.

CULTIVAR	AMMONIUM NITRATE	INOCULATION TREATMENT ( <u>Rhizobium</u> strains)		
		TAL 1000	TAL 236	TAL 309
		----- mg N/pot -----		
Early Bunch	405	582	175	467
Giza-4	461	625	381	249
Tato-34	462	452	130	276
Bachimba	394	312	265	229
Valencia R-2	315	496	133	248
Col. Correntino	379	309	288	288
156 of Cuba	503	331	345	106
Sulasmith	400	444	80	258
Blanco Rio Seg.	330	367	385	205
Egret	343	354	236	293
Makula Red	348	329	115	143
F3-261	184	239	191	134

<u>Source</u>	<u>d.f.</u>	<u>F</u>	
Cultivar	11	7.0**	LSD.05 for means = 137
Cultivar x strain	35	3.4**	



cultivar, Giza-4. Despite the presence of cultivar by strain interactions with these two cultivars, the percent that symbiotic yield is to mineral nitrogen yields were high for both Early Bunch and Giza-4.

Cultivars with low percentages of symbiotic to mineral N yields were those which consistently failed to exhibit a highly efficient symbiosis with any of the three strains. For example, 156 of Cuba had substantial early yield potential with  $\text{NH}_4\text{NO}_3$  yet its highest symbiotic yield (TAL 309) was only 62% of its mineral N yield. Makula Red is another example. Every strain treatment combination with this cultivar was lower than the average mean strain yield. Makula Red's potential with mineral N was high. Cultivar Sulasmith with a symbiotic percentage of 63%, had one very high strain treatment combination (TAL 1000) and two very low treatments.

It appears then, that a low symbiotic capacity, as defined in this experiment, can be the result of two phenomena. First, the cultivar can give low responses with all three effective strains. Second, the cultivar can have a more narrow rhizobial strain requirement for effectiveness than others in that it is effective with only one strain. Another example of this second case is that of Trifolium subterraneum cv. Woogenellup which has been shown to have more specific requirements for effectiveness than other cultivars of the same species (Gibson, 1964).

Results of Experiments 1, 2 and 4 also indicate the need to reconsider the process of screening strains of Rhizobium for effectiveness and to examine plant material released for commercial use for its symbiotic capabilities. Because of the specific and

complex interaction between strain and host it is dangerous to assume that a strain proven to be highly effective on one genotype will perform in a similar fashion with another genotype. This appears to hold true even when the strains were shown to be effective on more than one genotype. However strains which were effective on one genotype of peanut were always at least partially effective on the other cultivars. All three strains used in this experiment reduced acetylene with all cultivars tested.

Plant material to be released for commercial use should be examined for its symbiotic capacity relative to its genetic potential with mineral N, and the plant selection process should be done under conditions where the plant has to rely almost solely upon the symbiosis for its nitrogen requirements. In addition, it should be useful to grow genotypes with apparent low and high symbiotic capacity in the field (in the presence of a heterogeneous population of native rhizobia) to determine whether the genotypes will perform similarly to their performance in the greenhouse.

Data for the acetylene reduction assay (Appendix E) show almost no correlation between shoot yields and ethylene evolved. Early Bunch, a high fixing cultivar in this experiment, had significantly less activity than other cultivars. It is interesting to note that the two cultivars with the lowest apparent symbiotic capacity, 156 of Cuba and Makula Red, also had low nitrogenase activity levels. The fact that cultivars Early Bunch and Giza-4 showed significantly less activity than other cultivars indicates that some factor other than symbiotic efficiency was limiting nitrogen fixation at harvest. This

emphasizes the sensitivity of the acetylene reduction assay to other factors which limit growth.

The fact that the percentages that symbiotic yield are to yield of dry matter with  $\text{NH}_4\text{NO}_3$  were almost always less than 100, even when strains had passed through much previous selection for effectiveness, demonstrates the early advantage that a plant given mineral N has compared to a purely symbiotic plant. Similar percentages for N yield were closer to 100 than were the dry weight ratios. This was due to lower concentrations of N in plants given mineral N. Plants provided N probably experience factors other than N which were limiting to growth earlier than symbiotic plants and may have reached a physiological state where they could no longer take up  $\text{NH}_4^+$  ions efficiently (McElhannon and Mills, 1978).

In conclusion, this experiment emphasized the importance of the genotype-strain interaction on peanut. Some strains of Rhizobium such as TAL 1000 fix more nitrogen than other strains with a variety of peanut cultivars while some cultivars fix more nitrogen than others with a number of strains. The degree to which host by strain interactions are present points to the need to either: (1) solve the mysteries of invasive specificity such that there can be developed a single, maximally efficient Rhizobium for each plant type; (2) prove that selecting host genotypes for higher symbiotic capacity with a broad spectrum of strains can increase nitrogen fixation in peanut. Until the problems of invasive specificity are solved it seems probable, given the large numbers of peanut invasive Rhizobium in the tropics, that the most feasible method to ensure efficient symbiosis

with peanut would be to examine host genotypes for their symbiotic capacity. Selection for high symbiotic capacity could proceed during the selection process for other desirable agronomic characteristics.

## SUMMARY

The importance of peanut cultivation in the developing tropics is considerable. The peanut's prominence in international agricultural commerce means that it can be an important source of foreign exchange for developing countries. The attractiveness of peanut cultivation to small farmers is also enhanced because one expensive production input, nitrogen, is not required for this leguminous crop.

Peanuts planted in most tropical soils will encounter a considerable number of invasive native strains of Rhizobium. The presence of invasive soil strains is due to the fact that the peanut cross-inoculates with Rhizobium isolated from an extremely wide range of tropical legumes. Given the difficulties in overcoming native soil strains which compete with inoculum strains it seems plausible that selection of host types that are capable of fixing large amounts of nitrogen with many strains of Rhizobium may be a fruitful approach to increasing nitrogen fixation in the peanut-Rhizobium symbiosis. Most studies in the past have only examined the bacterium for effectiveness on one cultivar. This study was undertaken to examine the effect that the interaction between genotypes of peanut and strains of Rhizobium have on the symbiosis.

Two related greenhouse experiments examined a number of strains of Rhizobium for their effectiveness on two cultivars of peanut. Significant host by strain interactions were revealed in these experiments. Some strains produced high yields with one cultivar but failed to promote a highly efficient symbiosis with the other. This fact emphasized the danger in attempting to select superior strains

on only one cultivar. In addition, these results demonstrated the host's genetic role in efficient symbiosis. In each experiment a strain which was superior on both cultivars was identified. Because of their superior performance on two cultivars these strains were selected to test the symbiotic performance of twelve peanut cultivars in a third greenhouse experiment.

Results of this experiment showed that despite prior selection of strains for superior effectiveness on at least two cultivars, significant strain by cultivar interactions were present. Therefore, selection of strains on a number of cultivars does not result in identifying strains which will be highly effective on all other cultivars. There is specific genetic compatibility required between host and strain for efficient symbiosis. Strains to be used for inoculum production should therefore, be tested on each genotype for which the inoculum is intended.

Some cultivars in this experiment, on the average, fixed more nitrogen with these strains than others. The different average symbiotic yields of these cultivars were independent of the cultivars' yields when given  $\text{NH}_4\text{NO}_3$ . Therefore low yields of strain cultivar combinations were due to specific host strain incompatibility rather than lack of genetic vigor of the host. Just as no strain could be identified that was highly effective on every cultivar, there were no cultivars which consistently produced high yields with every strain.

Generally, a plant given mineral N yielded more dry matter than those relying on the symbiosis for nitrogen. Plants given mineral N

have an early advantage over symbiotic plants and are probably less sensitive to environmental stress.

A growth room study showed that the time required for nodule formation was both host and strain dependent. Cultivars with greater seed weights nodulated more slowly than those with smaller seeds. The time required for nodule formation was not related to the degree of symbiotic effectiveness of the host-strain combinations.

Effective symbiosis and time to nodule formation are both strain and host dependent. Whether cultivars which exhibited higher average symbiotic yields with the selected strains will perform in a similar manner in a field with a heterogeneous soil population remains to be determined. Cultivar performance in such fields would be a function of the interaction between the cultivar and the strains which formed the majority of nodules on the plant.

# APPENDIX A

TABLE 8. -- ANALYSIS OF VARIANCE FOR TABLES 1-7.

ANALYSIS OF VARIANCE FOR SHOOT N<sup>o</sup> OF TWO CULTIVARS OF  
PEANUT INOCULATED WITH FIVE STRAINS OF  
RHIZOBIUM SPP.

Source	d.f.	ss	ms	F
Total	56			
Block	3	556	185	.25
Treatment	13	874134	67241	90.4**
Strain	6	711722	118620	159.4**
Cultivar	1	109475	199475	147.1**
Strain x cul.	6	52937	8823	11.8**
Error	38*	28266	744	

\*adjusted for 1 missing pot

ANALYSIS OF VARIANCE FOR ACETYLENE REDUCTION OF  
TWO CULTIVARS OF PEANUT INOCULATED WITH  
FIVE STRAINS OF RHIZOBIUM SPP.

Source	d.f.	ss	ms	F
Total	55	4687		
Blocks	3	310	103	10.5**
Treatments	13	3993	307	31.2**
Cultivar	1	23	23	2.3
Strain	6	3863	644	65.4**
Cul. x strain	6	107	18	1.8
Error	38*	384	10	

\*adjusted for 1 missing pot



TABLE 8. -- ANALYSIS OF VARIANCE FOR TABLES 1-7.  
(continued)

ANALYSIS OF VARIANCE FOR SHOOT DRY WEIGHT OF  
TWELVE CULTIVARS OF PEANUT INOCULATED WITH  
THREE STRAINS OF RHIZOBIUM SPP.

Source	d.f.	ss	ms	F
Whole plot	143	2381		
Main plot	11	880	800	
Strain	3	834	278	
Strain x block	8	46	5.8	
Error (a)	0			
Cultivar	11	485	44.1	9.2**
Cultivar x strain	33	640	19.4	4.0**
Error (b)	78*	377	4.8	

\*adjusted for 10 missing pots

ANALYSIS OF VARIANCE FOR TOTAL SHOOT N OF TWELVE CULTIVARS OF  
ARACHIS HYPOGEA INOCULATED WITH THREE STRAINS OF  
RHIZOBIUM SPP.

Source	d.f.	ss	ms	F
Whole plot	143	2902059		
Main plot	11	987966		
Strain	3	990274		
Strain x block	8	97692		
Error (a)	0			
Cultivar	11	547343	49758	6.96**
Cultivar x strain	35	809389	24527	3.43**
Error (b)	78*	557361	7146	

\*adjusted for 10 missing pots

TABLE 8. -- ANALYSIS OF VARIANCE FOR TABLES 1-7.  
(continued)

ANALYSIS OF VARIANCE FOR THE RATIO OF SHOOT WEIGHT TO  
ROOT WEIGHT FOR TWO CULTIVARS OF PEANUT INOCULATED WITH  
FIVE STRAINS OF RHIZOBIUM SPP.

Source	d.f.	ss	ms	F
Total	55	145.8		
Blocks	3	.05	.02	.03
Treatments	13	127.7	9.8	20.1**
Cultivar	1	15.0	15.0	30.6**
Strain	6	107.8	18.0	36.7**
Cultivar x strain	6	4.9	.8	1.7
Error	38*	18.0	.5	

\*adjusted for 1 missing pot

ANALYSIS OF VARIANCES FOR THE SHOOT WEIGHTS OF  
TWO CULTIVARS OF PEANUT INOCULATED WITH SEVEN STRAINS OF  
RHIZOBIUM SPP. OR GIVEN  $\text{NH}_4\text{NO}_3$

Source	d.f.	ss	ms	F
Total	71	1016		
Blocks	3	35.4	11.8	6.0**
Treatments	17	890.0	52.4	26.5**
Cultivar	1	2.5	2.5	1.3
Strain	8	849.3	106.2	53.6**
Cultivar x strain	8	38.2	4.8	2.4*
Error	49*	91.2	1.98	

\*adjusted for 2 missing pots

TABLE 8. -- ANALYSIS OF VARIANCE FOR TABLES 1-7.  
(continued)

ANALYSIS OF VARIANCE FOR THE TIME TO NODULE FORMATION (TNF) FOR  
3 CULTIVARS OF PEANUTS INOCULATED WITH 10 STRAINS OF  
RHIZOBIUM SPP.

Source	d.f.	ss	ms	F
Total	127	1900		
Treatments	29	1612	55.6	19.0**
Strain	9	120	13.4	4.6**
Cultivar	2	728	364.2	124.3**
Strain x cultivar	18	764	42.4	14.5**
Error	98	288	2.9	

# APPENDIX B

TABLE 9. -- SHOOT DRY WEIGHT OF TWO CULTIVARS OF PEANUT INOCULATED  
WITH FIVE STRAINS OF RHIZOBIUM SPP.

Treatment	Florida Giant	Starr	Mean
	----- g/pot -----		
450 mg N	18.8 a	12.8 a	15.8 a
TAL 236	9.5 b	5.0 b	7.3 b
TAL 423	8.0 c	4.1 c	6.1 c
TAL 309	7.4 cd	4.0 c	5.7 c
TAL 170	6.9 d	4.4 bc	5.6 c
Uninoculated	7.7 cd	3.5 c	5.6 c
TAL 174	7.3 cd	3.8 c	5.6 c
Mean	9.4 a	5.4 b	

Means within a group not followed by the same letter are significant  
at  $P \leq .01$  as given by Duncan's New Multiple Range Test.

## APPENDIX C

TABLE 10. -- DAYS TO FIRST FLOWER OF TWO CULTIVARS OF PEANUT INOCULATED WITH FIVE STRAINS OF RHIZOBIUM SPP.

Treatment	Florida Giant	Starr	Mean
	----- days -----		
450 mg N	34.8	32.0	33.4 a
Uninoculated	35.0	33.5	34.3 a
TAL 170	35.3	33.5	34.4 a
TAL 309	34.8	34.3	34.5 a
TAL 423	36.5	34.3	35.4 a
TAL 236	37.0	34.0	35.5 a
TAL 174	37.0	35.0	36.0 a
Mean	35.8	33.8 b	

Means within a group not followed by the same letter are different at  $P \leq .01$ .

## APPENDIX D

TABLE 11. -- SUMMARY OF NODULE OBSERVATIONS, EXPERIMENT I

		TAL 170	TAL 423	TAL 236	TAL 174	TAL 309	+N	-N
% Effective Nodules	Florida Giant	100	0	95	0	95	0	0
	Starr	88	0	98	0	98	0	0
Nodule Number (Estimate)	Florida Giant	250	125	250	100	250	0	1
	Starr	250	250	250	250	250	0	4
Average Nodule Diameter (mm)	Florida Giant	2.1	1.0	1.6	.8	2.0	-	1.0
	Starr	1.4	1.0	1.5	1.0	1.4	-	1.5

## APPENDIX E

TABLE 12. -- ACETYLENE REDUCTION BY TWELVE CULTIVARS OF PEANUT INOCULATED WITH THREE STRAINS OF RHIZOBIUM SPP.

Cultivar	TAL 1000	TAL 236	TAL 309	Mean
COL. CORRENTINO	80.5	114.3	91.7	95.5 a
TATO-34	76.9	56.8	79.9	71.2 b
BLANCO RIO SEG.	80.8	71.4	47.1	66.4 bc
VALENCIA R-2	85.8	57.1	44.2	62.4 bc
BACHIMBA	51.1	57.1	79.9	61.5 bcd
GIZA-4	43.9	76.2	50.5	56.9 bcd
F3-261	44.9	55.9	53.6	51.5 bcd
EGRET	50.0	51.3	47.9	49.8 bcd
SULASMITH	70.5	22.2	56.5	49.7 bcd
MAKULA RED	51.3	41.0	43.5	45.3 cd
156 OF CUBA	20.1	63.6	49.6	44.4 cd
EARLY BUNCH	36.3	40.7	41.7	39.6 d
MEAN	57.7	59.0	56.8	

Means not followed by the same letter differ at  $P \leq .05$  as given by Duncan's New Multiple Range Test.

LSD for Cultivar Means = 19.6

LSD for Treatment Means= 33.8

## APPENDIX F

TABLE 13. -- SOURCE AND CHARACTERISTICS OF STRAINS USED IN EXPERIMENTS 1, 2, 3, AND 4

Strain Number	Source	Other Designations	Original Host	Reaction on BTB	Absorbtion of Congo Red	Comments
TAL 1000	NiftAL Project		A. hypogea	Alkaline	Medium	Recommended for peanut by NiftAL
TAL 236	"		"	"	"	Recommended for peanut by NiftAL. Widely used cowpea type Rhizobium.
TAL 309	"	CB756	Macotoloma africanum	"	Slight	
99 SU-ineff	R. Weaver, Texas A & M		A. hypogea	"	None	Ineffective mutant of a highly effective strain
T-1	"		"	"	Slight	Standard Texas peanut strain
TAL 170	NiftAL Project	176 A23	Vigna unguiculata	"	Heavy	
TAL 423	"	THA 304	Vigna radiata	"	"	
TAL 301	"		Acacia koa	"	None	
TAL 174	"	176 A32	Vigna unguiculata	"	Medium	
AH8	P. Singleton, U. of Hawaii		A. hypogea	"	Slight	
AH10	"		"	"	None	
UMKL 44	P. Somasagaran, NiftAL Project	TAL 651	Calopogonium muconoides	"	Slight	



## BIBIOGRAPHY

- Allen, O. N. and E. K. Allen. 1940. Response of the peanut plant to inoculation with Rhizobium, with special reference to morphological development of the nodules. *Botanical Gazette* 102:121-141.
- Bajpai, P. D., L. K. Lehri, and A. N. Pathak. 1974. Effect of seed inoculation with Rhizobium strains on the yield of leguminous crops. *Proc. Indian Natl. Sci. Acad., Part B*, 40(5):571-575.
- Baldwin, I. L. and E. B. Fred. 1929. Nomenclature of the root nodule bacteria of the leguminosae. *J. of Bact.* 17:141-150.
- Bohlool, B. B. and E. L. Schmidt. 1973. Persistence and competition aspects of Rhizobium japonicum observed in soil by immunofluorescence microscopy. *Soil Sci. Soc. of Am. Proc.* 37(4):561-64.
- Boyer, J. and G. Bond. 1942. The effectiveness of certain strains of the soya-bean nodule organism when associated with different varieties of the host plant. *Ann. Appl. Biol.* 29:103-8.
- Broughton, W. J. and M. J. Dilworth. 1971. Control of leghemoglobin synthesis in snakebean. *Biochem. J.* 125:1075-80.
- Brower, R. 1962. Nutritive influences on the distribution of dry matter in the plant. *Neth. J. Agric. Sci.* 10(5):399-408.
- Caldwell, B. E. and G. Vest. 1968. Nodulation interaction between soybean genotype and serogroups of Rhizobium japonicum. *Crop Sci.* 8:680-682.
- Caldwell, B. E. and G. Vest. 1970. Effects of Rhizobium japonicum strain on soybean yields. *Crop Sci.* 10:19-21.
- Dadarwal, K. R. 1974. Nodulation and serological studies of Rhizobia from six species of Arachis. *Plant Soil* 40(3):535-544.
- Dart, P. J. 1974. The infection process. In A. Quispel (ed.) *The Biology of Nitrogen Fixation*. North Holland Publishing Co., Amsterdam.
- Diatloff, A. and J. Brockwell. 1976. Symbiotic properties of Rhizobium japonicum and competitive success in nodulation of two Glycine max cultivars by effective and ineffective strains. *Aust. J. Exp. Agric. An. Husb.* 16:514-521.
- Diatloff, A. and S. Langford. 1975. Effective natural nodulation of peanuts in Queensland. *Queensland Journal of Agricultural and Animal Sciences* 32(1):95-100.

- Duhigg, P., B. Melton, and A. Baltensperger. 1978. Selection for acetylene reduction rates in 'Mesilla' alfalfa. *Crop Sci.* 18(5): 813-17.
- El-Sherbeeney, M. H., D. A. Lawes, and L. R. Mytton. 1977. Symbiotic variability in Vicia faba 2. Genetic variation in Vicia faba. *Euphytica*, 26(2):377-383.
- Gaur, Y. D., A. N. Sen, and N. S. Subba Rao. 1974. Promiscuity in groundnut Rhizobium association. *Zentralbl Bakterial Parasit Infectiowaki Hyg., Zweite Naturwi Ss Abt.* 129(3/4):369-372.
- Gibson, A. H. 1962. Genetic variation in the effectiveness of nodulation of lucerne varieties. *Aust. J. Agric. Res.* 13:388-399.
- Gibson, A. H. 1964. Genetic control of strain-specific ineffective nodulation in Trifolium subterraneum L. *Aust. J. Agric. Res.* 15:37-49.
- Gibson, A. H., R. A. Date, J. A. Ireland, and J. Brockwell. 1976. A comparison of competitiveness and persistence amongst five strains of Rhizobium trifolii. *Soil Biol. Biochem.* 8:395-401.
- Graham, P. H. 1976. Identification and classification of root-nodule bacteria. In P. S. Nutman (ed.) *Symbiotic Nitrogen Fixation in Plants*. Cambridge University Press, Cambridge.
- Habish, H. A. and S. M. Khairi. 1968. Nodulation of legumes in the Sudan: cross inoculation groups and the associated Rhizobium strains. *Exp'l. Agric.* 4:227-234.
- Hardy, R. W. F., R. D. Holsten, E. K. Jackson, and R. C. Burns. 1968. The acetylene-ethylene array for  $N_2$  fixation. *Plant Physiol.* 43:1185-1207.
- Hardy, R. W. F. and U. D. Havelka. 1976. Photosynthate as a major factor limiting nitrogen fixation by field-grown legumes with emphasis on soybeans. In P. S. Nutman (ed.) *Symbiotic nitrogen fixation in plants*. Cambridge University Press, Cambridge.
- Hely, F. W. 1957. Symbiotic variation in Trifolium ambiguum M. Bieb. with special reference to the nature of resistance. *Aust. J. Biol. Sci.* 10:1-16.
- Hickey, J. M., W. K. Robertson, D. H. Hubbell, E. B. Whitty. 1974. Inoculation, liming, and fertilization of peanuts on Lakeland fine sand. *Proc. Soil Crop Sci. Soc. Fla.* 33:218-222.
- Johnson, H. W. and Ura Mae Means. 1960. Interaction between genotypes of soybeans and genotypes of nodulating bacteria. *Agron. J.* 52:651-654.

- Jones, D. G. 1963. Breeding for nodule characteristics in white clover. Rep. Welsh Plant Breeding Station for 1963:43-45.
- Lie, T. A., D. Hille, R. Lambers, and A. Houwers. 1976. Symbiotic specialization in pea plants: some environmental effects on nodulation and nitrogen fixation. p. 319-333. In P. S. Nutman (ed.) Symbiotic nitrogen fixation in plants. Cambridge University Press, Cambridge.
- McElhannon, W. S. and H. A. Mills. 1978. Influence of percent  $\text{NO}_3/\text{NH}_4$  on growth, N absorption, and assimilation by lima beans in solution culture. Agron. J. 70(6):1027-32.
- Mitchell, H. L. 1972. Microdetermination of nitrogen in plant tissues. J. AOAC. 55(1):1-3.
- Nutman, P. S. 1946. Genetical factors concerned in the symbiosis of clover and nodule bacteria. Nature 157:463.
- Nutman, P. S. and M. P. Read. 1952. Symbiotic adaption in local strains of red clover and nodule bacteria. Pl. Soil 4:57-75.
- Nutman, P. S. 1961. Variation in symbiotic effectiveness in subterranean clover (Trifolium subterraneum L.). Aust. J. Ag. Sci. 12:212-226.
- Nutman, P. S. 1967. Varietal differences in the nodulation of subterranean clover. Aust. J. Agric. Res. 18:381-425.
- Pinto, C. M., P. Y. Yao, and J. M. Vincent. 1974. Nodulating competitiveness amongst strains of Rhizobium meliloti and R. trifolii. Aust. J. Agric. Res. 25:317-329.
- Rachie, K. O. 1974. Grain legumes of the lowland tropics. In Advances in Agronomy 26:1-132. Am. Soc. Agron., Madison, Wis.
- Robinson, A. L. 1969. Host selection for effective Rhizobium trifolii by red clover and subterranean clover in the field. Aust. J. Ag. Res. 20:1053-1060.
- Roughley, R. J., W. M. Blowes, and D. F. Herridge. 1976. Nodulation of Trifolium subterraneum by introduced Rhizobia in competition with naturalized strains. Soil Biol. Biochem. 8:403-407.
- Sandman, W. P. L. 1970. Results of formal greenhouse tests (1963-1969) of strains of Rhizobium. Rhodesian Ministry of Agriculture G. D. S39494-221.
- Seetin, N. W. and D. K. Barnes. 1977. Variation among alfalfa genotypes for rates of acetylene reduction. Crop Sci. 17:783-787.

- Smartt, J. 1976. Tropical pulses. Longman Group Ltd., London.
- Snedecor, G. W., and W. G. Cochran. 1974. Statistical methods. Iowa State University Press, Ames, Iowa.
- Subba Rao, N. S. 1976. Field response of legumes in India to inoculation and fertilizer application. In P. S. Nutman (ed.) Symbiotic nitrogen fixation in plants. Cambridge University Press, Cambridge.
- Thompson, J. A., R. J. Roughley, and D. F. Herridge. 1974. Criteria and methods for comparing the effectiveness of Rhizobium strains for pasture legumes under field conditions. Plant and Soil 40:511-524.
- U.N.F.A.O. 1973. The impact of urbanization on food demand. Monthly Bulletin of Agricultural Economics and Statistics 22.
- Van der Merwe, S. P., B. W. Strijdom, and C. J. Uys. 1974. Groundnut response to seed inoculation under extensive agricultural practices in South African soils. Phytophylactica 6(4):295-301.
- Vincent, J. M. 1970. A manual for the practical study of root-nodule bacteria. Burgess and Son Ltd., Berkshire, G. B.
- Vincent, J. M. 1974. Root-nodule symbiosis with Rhizobium. In A. Quispel (ed.) The Biology of Nitrogen Fixation. North Holland Publishing Co., Amsterdam.
- Vincent, J. J. and L. M. Waters. 1953. The influence of the host on competition amongst clover root-nodule bacteria. J. Gen. Microbiol. 9:357-370.
- Weaver, R. W. 1972. A new technique for most-probable number counts of Rhizobia. Plant and Soil 36:219-222.
- Weaver, R. W. 1974. Effectiveness of Rhizobia forming nodules on Texas grown peanuts. Peanut Sci. 1:23-25.
- Weaver, R. W. 1975. Growing plants for Rhizobium effectiveness tests. Soil Biol. Biochem. 7:77-78.
- Weber, D. F. and V. L. Miller. 1972. Effect of soil temperature on Rhizobium japonicum serogroup distribution in soybean nodules. Agron. J. 64:796-799.
- Williams, L. F. and D. L. Lynch. 1954. Inheritance of a non-nodulating character in the soybean. Agron. J. 46:28-29.